EFFECTS OF DRYING METHODS ON THE ANTIOXIDANT ACTIVITIES ON *BENINCASA HISPIDA* SEED EXTRACTS

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ABSTRACT

The present study investigated the changes in total phenolic content (TPC) and antioxidant capacity of *B.hispida* seeds after three drying treatment (sun drying, oven drying and freeze drying) compared to fresh samples by DPPH radical scavenging method and total phenolic content. Ethanol is used as the solvent for extraction because ethanol can obtain the highest antioxidant activity due to its highest polarity. The analysis was conducted in University Malaysia Pahang laboratory using the ultraviolet-visible spectrophotometry. The results of this study showed that the water content, antioxidant activity and total phenolic content which are highest from *B.hispida* seeds were obtained by freeze drying. Sun drying was the worst in antioxidant capacity and phenolic content because the value obtained are less compared other methods. Overall, *B. hispida* may have high potential applications in the medical and food industries.

KESAN-KESAN KAEDAH PENGERINGAN TERHADAP AKTIVITI ANTIOKSIDAN KE ATAS PENGEKSTRAKAN BIJI *BENINCASA HISPIDA*

ABSTRAK

Kajian ini menyiasat perubahan dalam jumlah kandungan phenol dan kapasiti antioksidan dalam biji benih *B.hispida* selepas tiga kaedah pengeringan (pengeringan matahari, pengeringan ketuhar dan pengeringan sejukbeku) berbanding dengan sampel segar dengan kaedah DPPH radikal dan kandungan phenolik total. Etanol digunakan sebagai pelarut untuk pengekstrakan kerana etanol boleh mendapatkan aktiviti antioksidan tertinggi disebabkan kepolarannya adalah tinggi. Analisis telah dijalankan di makmal Universiti Malaysia Pahang dengan menggunakan spektrofotometri ultraungu. Keputusan kajian ini menunjukkan bahawa kandungan kelembapan, aktiviti antioksida dan kandungan jumlah phenol yang tertinggi telah didapati dalam biji benih *B.hispida* adalah daripada pengeringan sejukbeku. Pengeringan matahari adalah yang terburuk dalam kapasiti antioksidan dan kandungan phenolik kerana mempunyai jumlah yang sedikit. Secara keseluruhan, *B. hispida* mungkin mempunyai aplikasi potensi tinggi dalam industri perubatan dan makanan.

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LIST OF ABBREVIATIONS

AAE	Ascorbic acid equivalent
BH	*Benincasa hispida*
DPPH	1,1-diphenyl-2-picrylhydrazyl radical
DW	Distilled water
TAE	Tannic acid equivalent
TPC	Total phenolic content

CHAPTER 1

INTRODUCTION

1.1 Research Background

Nowadays, oxidation is essential to many living organisms for the production of energy to fuel biological process. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, genetic damage and the promotion of disease and aging. These free radicals are unstable and it can react with cells and destroy it. In order to reduce oxidation damage to the human, it is essential to develop and utilize effective natural antioxidants to protect the human body from free radicals and reduce risk of many diseases, cancer, arthritis and the aging process (Nandita *et al*., 2004).

As the alternative methods to reduce oxidation damage to the human, many of researchers do some research about the foods. Most of the foods high in antioxidants come from fruits and vegetables. Natural antioxidant from fruit is safer for human body because it is come from natural condition compared with the synthetic antioxidant (Weerasak *et al.*, 2008). According to an old Korean medical encyclopedia, the "Donguibogam" the *Benincasa hispida* is a good source of valuable nutrients including organic acids, natural sugars, amino acids, vitamin and mineral elements. Furthermore, *B.hispida* or Kundur fruit is one of the fruit vegetables currently gaining popularity among researchers due to its potential health benefits because of high nutritional value and functional properties such as antioxidant, anti-ulcer, anti-diarrheal and anti-angiogenic.

Benincasa hispida is mainly cultivated in China, and its seeds are obtained from the insideof dried, matured *B.hispida*, which is harvested during the months of August and September (Ji and Lee, 1988). Researchers from Taiwan found seeds have a higher capacity on anti-oxidation and inhibition of angiotensin-converting enzyme (ACE) activity than the fresh. It may be due to its high phenolic contents and superoxide dismutase activity (Huang *et al*., 2004). Thus, they believe, seed extracts of dong gua may offer a good health benefits on lowering risk of cardiovascular diseases and cancers. The seed extract is a good source of carbohydrate, amino acids, proteins and phenolic compounds. The antioxidant activity of the plant extract was affected by extraction solvents. Common solvents such as acetone, methanol, ethanol, water, hexane, chlorofoam, butanol and petroleum ether were used to extract antioxidant contained in fruits (Mohsen and Ammar, 2009).

Furthermore, the seasonal variations of plants can be eliminated using dried seeds and it can be kept for a long period without any damange. Seeds of *B.hispida* are generally dried to low moisture content for long-storage. Rapid and efficient drying techniques and methods that could minimize the nutrient loss have acquired considerable attention (Afzan *et al*., 1999), although dehydration is probably the oldest method of food preservation practiced by mankind (Antonio *et al*., 2008). Drying enhanced antioxidant activity by the formation of phenolics compounds. Phenolic compounds play a key contribution to the antioxidant property in dried of *B.hispida* seeds.

1.2 Statement of Problem

Fruits or herbs often require drying after harvested because they contained high moisture content which was the main factor contributed to the spoilage of highly perishable of fruits (Muller *et al*., 1989). Therefore, drying could improve shelf life, encapsulate original flavour, reduce storage volume and maintain nutritional values of fruits if compared to the fresh fruits (Gunhan *et al*., 2005). The antioxidant activity of the fruit was found to be reduced after dried under sun, oven or freeze dried (Chan *et al*., 2009). The degree of reduction of antioxidant activity in different drying methods was found to vary with different drying temperature and time (Katsube *et al*., 2008).

Thus, it is necessary to determine what effect drying has on the antioxidant activity of *B.hispida*. Michalczyk, Macura and Matuszak (2009) reported that freeze drying is much more effective in preserving valuable food compunds that traditional methods of drying.

However, information on changes in antioxidant activity of seeds *B.hispida* after drying is limited. For this reason, the present study investigated the effects of drying methods (oven drying, sun drying and freeze drying) on the antioxidant activity for seeking the potential drying process for the extracted from the seeds of *B.hispida*.

1.3 Research Objective

The main objective of this research project is to assess the effects of different drying method (oven dried, sun dried and freeze dried) on the antioxidant activity of the seeds extracted from *Benincasa hispida*.

1.4 Scope of Proposed Study

In order to achieve the research objectives, there are a few scopes of work that have been identified as follows:

1. Study on the effects of different drying methods on moisture content.

2. Determination of antioxidant activities from different drying method.

1.5 Significant of Proposed Study

Natural antioxidants are perceived safe, less toxic and beneficial for human health. However, it is very expensive and not widely commercialized. Source of antioxidants such as spices andherbs, and such material have been used troughout history for flavouring and preservative (Kikuzaki and Nakatani, 1993). The purposes why needed to find the alternative sources of natural antioxidants such as:

1. To make sure the price is low and value to commercialize.
2. Demand is very high, so it very easy for the antioxidant processes industry to get profit and market.
3. Dried of natural antioxidants may enhanced of antioxidant activity by the formation of phenolics compounds.
4. Reduce the energy consumed and cost of production with drying of natural antioxidants (Fatouh *et al*., 2006).

CHAPTER 2

LITERATURE REVIEW

2.1 Oxidation

Free radical is naturally produced in our body by the metabolism of amino acids and fats. Oxidation is essential to many living organism for the production of energy to fuel biological process. However the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid, arthritis, and arteriosclerosis as well degenerative processes associated with aging (Halliwel and Gutteridge, 2003). These free radicals are unstable and it can react with cells and destroy it. The bind of free radical with DNA structure will lead to mutation and it is cause of cancer. Free radicals formed in our body in several of type such as superoxide, hydroxyl, peroxyl and alkoxy.

To overcome this problem, the antioxidant is needed to inhibit all the free radical from react. They scavenge radicals by inhibiting initiation and breaking of chain reaction, suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide and quenching superoxide and single oxygen (Shi *et al*., 2001)

2.2 *Benincasa hispida*

2.2.1 The Morphology of Seeds *B.hispida*

The seeds of *Benincasa hispida* are defined as the seed and nutshell of *Benincasa hispida* Cogniaux, which belongs to a family of Cucurbitaceae, according to International Plant Name Index (IPNI) and the Korea Pharmacopoeia, the book of standard oriental medicine and herbal science (Ji and Lee, 1988). *Benincasa hispida* is one of the species of cucurbit family, which has a great potential for functional food production. One of the unique characterictics of Kundur fruit, if there is no injury to the fruits, in that it can be stored for many months, even for a full year in dry and cool atmospheres (Morton, 1971).

Kundur fruit is easier to grow than any of other cucurbits and mainly cultivated in China, and it seeds are obtained from the inside of dried, matured *B.hispida,* which is harvested during the months of August and September (Ji and

Lee, 1988). The seeds of Kundur fruit are flat, smooth and buff ranging from 1.0 to 1.5 cm in length and 0.5 to 0.8 cm in width, depending on the type and shape of fruit (Raveendra and Martin, 2006). White or yellowish white seeds (Figure 2.1) are filled in the center of the fruit (Morton, 1971). From Figure 2.2, as the fruit matures, the seeds color changes from white to yellowish brown. The seeds, which also have considerable amount of oil, can be fried and used as a snack.

Figure 2.1 Half cut Kundur (*Benincasa hispida*) fruit

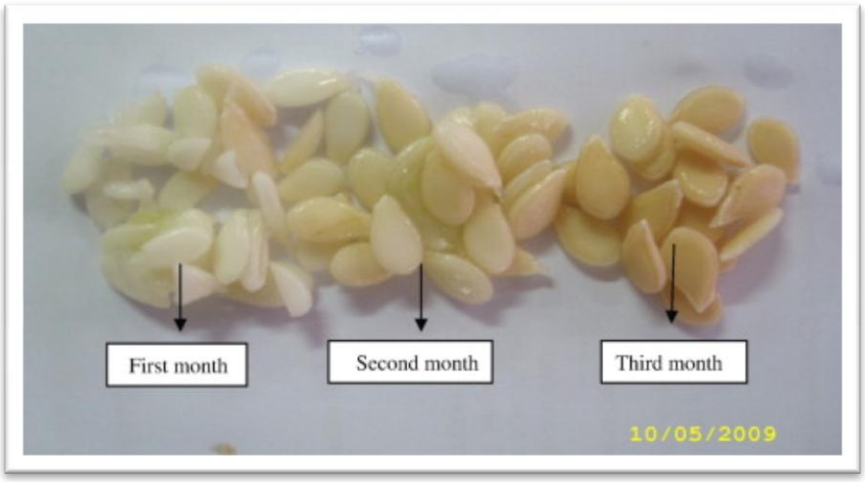

Figure 2.2 Color changes in Kundur (*Benincasa hispida*) fruit seed with maturity

2.2.2 The Uses and Benefits of *B.hispida* Seeds.

Due to high nutritional value and its growing demand, it could be suggested that Kundur fruit might be explored for uses in different food commodities such as jams, juices, beverages, cakes and ice cream for value-addition. Although some preliminary data on nutritional value of Kundur fruit is available, there is lack of information especially on nutritional profile of other parts of Kundur fruits such as seeds, skin and core which could be investigated. Especially, there is much need to characterize the Kundur fruit seeds. Lee *et al*.(2005) found that there is a potential angiogenic inhibitor in Kundur fruit seeds against tumor growth and obesity. Besides, Morton (1971) reported that the seed and seed oil have been in use to expel tapeworms. The seeds also help to reduce internal heat of body in summer and very useful in kidney stones. The seeds which also have considerable amount of oil, can be fried and used as a snack. Furthermore, the seed extract of *Benincasa*

hispida facilitates mucus secretion and also prevents gastrics ulcer (Grover *et al*., 2000).

2.2.3 Previous Studies Done on *B.hispida* Seeds.

Mingyu *et al.* (1995) studied about the different parts of Kundur fruit such as pulp, seed and skin stated that protein and free amino acids are present in high amounts in seed, with 5714.017 and 264.366 mg/100 g fresh weight. Total protein and free amino acids are the lowest in amount in the pulp, having concentrations of 216.400 and 92.549 mg/100 g fresh weight.Beside that, Grover *et al.* (2001) stated that *Benincasa hispida* seeds are mainly composed of saponin, urea, citrulline, linoleic acid, oleic acid and fatty acids. The seeds also contains minute amounts of a triterpenoid known as isomultiflorenol, proteins such as cucurbitacin B (Uchikoba *et al*., 1998).

Regarding the chemical composition of Kundur fruit seed, Martin (1984) suggested that Kundur fruit seed is perhaps one of the best cucurbit seed oil sources for the hot and humid tropics. It is further supported by Sew *et al.*(2010) that Kundur seed oil contains a high proportion of an essential fatty acid, linoleic acid, accounting for 67.37% of the total fatty acids. The oil from the seeds is soporific, good for the brain and liver, and also useful in the treatment of syphilis (Qadrie *et al*., 2009).

2.3 Natural Antioxidants From Plants

2.3.1 Source of Natural Antioxidants

Natural antioxidant, particularly in fruits and vegetables have gained interestamong consumers and the scientific community because epidemiological studies haveindicated that frequent consumption of natural antioxidant is associated with a lowerrisk of cardiovascular disease and cancer (Renaud *et al*., 1998). During this recent year, the use of fruit and vegetable juice has been increasing due to their health benefit to human beings. Fruits are rich with antioxidant that helps in lowering incidence of degenerative disease such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and acceleration of the ageing process (Feskanich *et al*., 2000; Gordon, 1996; Halliwell, 1996). This alternative sources of naturalantioxidants are needed to make sure the price is low and value to commercialize.

Antioxidant is defined as the molecule that can slow or prevent theoxidation process of other molecules. To get the natural antioxidants we should extract it from fruits or vegetables first. For those who need the antioxidant they can get it through the fruit when they eat. But for the food and pharmaceutical industries, the extract of antioxidants from fruits and vegetables is needed for their manufacturing process. The natural antioxidants can be extracted through the fruits, vegetables, spies and herbs. Seeds of *Benincasa hispida* is the material for the purpose ofextraction. Before extraction process, seeds of *B.hispida* must be dried first to low moisture of content.

Dried seeds from B.hispida could be used as a source of natural antioxidants in the food industry.

A study by Huang *et al*. (2004), on the in vitro antioxidant activity of Kundur fruit demonstrated that the seed has the highest capacity for inhibition of linoleic acid oxidation and scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals compared to the peel, pulp and core of the fruit. This may be due to higher total phenolic contents and superoxide dismutase (SOD) activity of the seeds. In addition, the fruit may provide protective effects against the development of atherosclerosis and also exhibits anticarcinogenic effects in vitro (Huang *et al*., 2004).

Beside that, Gill *et al*. (2010) investigated the anti-inflammatory effects of Kundur fruit seed. He suggested that free radical scavenging activity of Kundur fruit seed might have been responsible for the reduction of inflammation in the carrageenan-induced paw edema in rats. Some research about the common medicinal and pharmacological properties of different parts of Kundur (*Benincasa hispida*) fruit have also been summarized in Table 2.1.

Table 2.1 Some common medicinal and pharmacological properties of different partsof Kundur (*Benincasa hispida*) fruit

Part	Medicinal and pharmacological properties.	References
Pulp	Anti-inflammatory, anti-ulcer, anti-depressant, anti-histaminic, antioxidant, used for Alzheimer disease treatment	Grover and Rathi (1984), Mingyu *et al*.(1995), Huang *et al*.(2004)
Seed	Anti-angiogenic, anti-tumor, antioxidant, beneficial effects for brain and liver, used for the treatment of syphilis, cardiovascular diseases and softening or soothing the skin.	Qadrie *et al*.(2009), Lee *et al*.(2005), Huang *et al*. (2004)
Peel	Antioxidant activity, inhibition of angiotensin converting enzyme (ACE)	Huang *et al*. (2004)

Reference: Zaini *et al.* (2011)

2.3.2 Benefits of Antioxidants

The interest in studying antioxidant activity had increased recently due to the increased public awareness on the benefits of antioxidant in disease prevention (Kaefer and Milner, 2008). Beside that, oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants will terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols.

Furthermore, natural antioxidants are widely used in the pharmaceutical and food processing. Antioxidants play a significant role to prevent many several diseases like Alzheimer disease, cancer, and even aging. As we know that natural